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SESQUITERPENE LACTONES AND FLAVONOIDS FROM *Artemisia albida*

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The five known lactones matricarin, austricin, canin, and achillin guaianolides and argolide germacranolide and the two flavonoids eupatilin and its 7-O-methyl ester were isolated for the first time from the aerial part of Artemisia albida *Willd. The structure of eupatilin was confirmed by an x-ray structure analysis.*

Key words: *Artemisia albida* Willd., guaianolides, germacranolides, flavonoids, eupatilin, x-ray structure analysis.

Artemisia albida Willd. grows in East-Kazakhstan and Akmolinsk Regions of the Republic of Kazakhstan and in the Altai [1]. Its chemical composition has not previously been studied.

Aqueous extraction [2] of the air-dried aerial part of the plant and subsequent chromatography of the obtained resin over silica gel isolated successively known sesquiterpene lactones that were identified as matricarin [3], austricin [4], canin [5], argolide [6], and achillin [7] by comparison of their TLCs with those of authentic specimens and by their PMR spectra.

Furthermore, we isolated two compounds as yellow crystals. High-resolution mass spectrometry established their empirical formulas as $C_{18}H_{16}O_7$ and $C_{19}H_{18}O_7$. The PMR spectra are consistent with trimethoxydihydroxy- and tetramethoxyhydroxyflavones, respectively. The second one is the *O*-methyl ester of the first.

These spectra show that both flavonoids have the same 3′,4′-disubstituted phenyl ring. One hydroxyl in both molecules is located on C-5 (narrow singlet for spectra recorded in DMSO-d_6 solution). Signals for two aromatic H atoms in their bicyclic part also appear as narrow singlets. This indicates unambiguously that one H is on C-3; the other, in one of three positions of the benzpyrone fragment of both molecules, C-6, C-7, or C-8.

Next it is important to note in comparing these two PMR spectra that one of the singlets has almost the same position and a second is shifted noticeably (by 0.4 ppm) to weak field on going from the spectrum of the flavonoid $C_{18}H_{16}O_7$ to that of $C_{19}H_{18}O_7$. This fact places the second OH of $C_{18}H_{16}O_7$ on C-7 and the second H atom of the benzpyrone fragment on C-8. Thus, this flavonoid has structure **1** and the second ($C_{19}H_{18}O_7$) is its 7-O-methyl ester **2**. The PMR spectrum of the latter has a characteristic narrow singlet for the 5-OH hydroxyl proton. In fact, the x-ray structure analysis (XSA) (Fig. 1) confirmed the correctness of the interpretation of the PMR data and the proposed structure **1**.

In general the molecular geometry is typical for this class of compounds. The chromene skeleton is planar within ±0.02 Å. The deviations of the O atoms bonded to it are <0.06 Å but reach -0.18 Å for O4. The mean-square deviation of all nonhydrogen atoms in **1** (except for C-15) is 0.065 Å. The C-15 methyl is out of this plane toward the α-side by 0.81 Å. The torsion angle C15O4C6C5 is 69.6(3)°. The plane passing through the phenyl ring is planar within 0.01 Å. The deviations of the methoxyls bonded to the aromatic ring are insignificant and reach 0.15 Å only for C-16. The angle between the planes of the main skeleton and the phenyl group is 4.2°. The torsion angle O1C2C9C14 is -2.2°.

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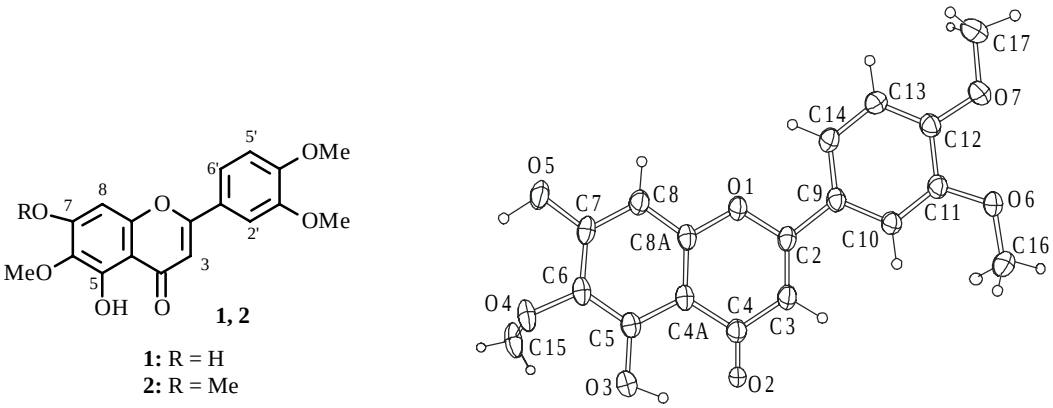

Fig. 1. Flavonoids **1**, **2** and molecular structure of **1**.

Bond lengths and angles in the structure are close to the normal values [8] and those expected for flavonoids [9, 10]. The exceptions are the bond angles around C-2, the values of which vary from 111.3(2) (O1C2C9) to 127.5(2)° (C3C2C9) and are also slightly different than the standard angle C3C4C4a [115.3(2)°]. The distortion of the bond angles around C2 is a rare phenomenon for flavonoids [10].

The rather strong intramolecular H-bond O3–H...O2 [O–H 0.92(3), H...O 1.75(3), O...O 2.590(2) Å, O–H...O 150(3)°] is noteworthy. Molecules of **1** in the crystal are bound in infinite chains by H-bonds O5–H...O2 [O–H 0.99(3), H...O 1.90(3), O...O 2.734(2) Å, O–H...O 140(3)°]. The chains are joined into layers through π-stacking interactions between phenyl and benzene/pyrone rings. The corresponding distances between centroids and planes are 3.713(1)/3.831(1) and 3.5/3.4 Å.

Flavonoid **1** is known and was first described under the name eupatilin as the cytotoxic component of leaves of *Eupatorium semperviratum* DC [11]. Later it was found in the aerial part of *A. frigida* Willd. [12], *A. asiatica* Nakai [13], and *Serifidium santolinum* Poljak (Compositae) [14]. Flavonoid **2** was previously found in *A. lanata* [15] and *A. assoniana* [16] and was first prepared by chemical modification of natural flavonoids [11, 17].

The quantitative contents of sesquiterpene lactones and flavonoids in the studied material were determined by HPLC using pure specimens of these compounds. It was found that the contents of matricarin, austricin, canin, argolide, achillin, and flavonoids **1** and **2** were 0.04, 0.57, 0.024, 0.039, 0.0014, 0.01, and 0.102%, respectively.

EXPERIMENTAL

Melting points were determined on a Boetius apparatus. PMR spectra were recorded on a Bruker DRX-500 spectrometer (working frequency 500.13 MHz for ^{1}H). High-resolution mass spectra (EI, 70 eV) were obtained in a Finnigan MAT 8200 instrument; IR spectra, on an Avatar 360 instrument (Thermo Nicolet).

Column chromatography was carried out over KSK silica gel with a compound:sorbent ratio of ~1:20. TLC used Silufol plates with elution by petroleum ether:ethylacetate (1:1) and development by saturated aqueous $KMnO_4$ and saturated aqueous $FeCl_3$.

HPLC was performed in a Hewlett Packard Agilent 1100 instrument under the following conditions: analytical column, Zorbax CB-C_{18} sorbent (150 × 4.6 mm), 5 μm, mobile phase CH_3OH:H_2O (50:50) and CH_3CN:H_2O (50:50), detection at 254 nm, column temperature ambient, mobile-phase flow rate 0.5 and 0.3 mL/min, sample volume 20 μL.

Quantitative analysis was performed using the areas of the chromatographic peaks and pure standard compounds isolated from the same raw material.

The XSA was performed on a Bruker P4 diffractometer (Mo Kα-radiation, graphite monochromator, 2θ/θ-scanning at 2θ < 52°). The structure was solved by direct methods using the SHELXS-97 program. The structure factors were refined by anisotropic-isotropic (for H atoms) full-matrix least-squares methods using the SHELXL-97 program. The positions of H atoms were found from difference syntheses. Absorption corrections were applied by integration over the crystal facets (transmission 0.94-0.99).

Starting raw material (aerial part of *A. albida* Willd.) was collected near Ivanovskii ridge of East-Kazakhstan Region in August 2004 during budding, dried in air, and ground before extraction.

Isolation of Components from Raw Material. A weighed portion of raw material (4.7 kg) was extracted three times with water at 80-90°C. The extract was cooled to room temperature, filtered, and extracted three times with $CHCl_3$. The extract was evaporated to dryness. The resulting resin (96 g) was separated by column chromatography over SiO_2 with elution by benzene and then benzene:ethylacetate mixtures with an increasing fraction of the latter. Matricarin and argolide crystallized from the fraction eluted by benzene:ethylacetate (9:1); then achillin and flavonoid **2**, from the 5:1 fraction; austricin, 4:1; canin, 10:9; and flavonoid **1**, 3:7.

7-O-Methyl Ester of Eupatilin (2). Yellow crystals, mp 153-154°C (ethylacetate), lit. [11] mp 190-191°C (benzene:hexane).

IR spectrum (KBr, ν, cm^{-1}): 2943, 1766, 1661, 1632, 1590, 1517, 1495, 1459, 1361, 1269, 1121, 1018. Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 358 (100) $[M]^+$, 343 (96) $[M - Me]^+$, 312 (32) $[M - Me - MeO]^+$, 181 (18), 163 (20), 153 (41), 148 (10), 125 (6), 69 (23), 53 (6), 28 (4). Found, *m*/*z*: 358.10091 $[M]^+$, $C_{19}H_{18}O_7$.

PMR spectrum (500 MHz, DMSO-d_6, J/Hz): 3.76, 3.86, 3.89, 6.60 (3H each, singlets, OMe × 4), 6.96 (1H, s, H-3), 7.01 (1H, s, H-8), 7.20 (1H, d, J = 8, H-5′), 7.82 (1H, d, J = 2, H-2′), 7.71 (1H, dd, J = 8.2, H-6′), 10.6 (1H, s, C5-OH).

Eupatilin (1). Yellow crystals, mp 236-239°C (ethylacetate), lit. [11] mp 234-236°C (ethylacetate).

IR spectrum (KBr, ν, cm^{-1}): 3002, 1652, 1620, 1588, 1577, 1511, 1465, 1425, 1375, 1335, 1264, 1216, 1148, 1024, 993, 839, 816, 771, 576. Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 344 (88) $[M]^+$, 329 (60) $[M - Me]^+$, 326 (58) $[M - H_2O]^+$, 301 (49), 298 (12), 167 (11), 163 (28), 153 (11), 139 (16), 111 (100), 91 (12), 69 (47), 43 (83), 41 (15). Found, *m*/*z*: 344.09320 $[M]^+$, $C_{18}H_{16}O_7$.

PMR spectrum (500 MHz, DMSO-d_6, J/Hz): 3.76, 3.83, 3.86 (3H each, singlets, OMe × 3), 6.60 (1H, s, H-8), 6.89 (1H, s, H-3), 7.07 (1H, d, J = 8.0, H-5′), 7.50 (1H, d, J = 2, H-2′), 7.61 (1H, dd, J = 8.2, H-6′), 10.06 (1H, br.s, C8-OH), 13.02 (1H, s, C5-OH).

XSA. Crystals of **1** are monoclinic, $a = 13.081(3)$, $b = 8.7834(15)$, $c = 15.345(3)$ Å, $\beta = 113.737(16)°$, $V = 1613.9(5)$ $Å^3$, space group $P2_1/n$, $C_{18}H_{16}O_7$, $Z = 4$, $d_c = 1.417$ g/cm^3, dimensions 0.1 × 0.8 × 0.8 mm^3, $wR_2 = 0.1408$, S = 1.04 for all 3171 reflections, R = 0.0465 for 2426 reflections with $I > 2\sigma$.

The XSA results were deposited as a CIF file in the Cambridge Crystallographic Database (CCDC 284179).

REFERENCES

1. N. V. Pavlov, *Flora of Kazakhstan* [in Russian], Nauka, Alma-Ata (1966), Vol. 8.
2. K. S. Rybalko, *Natural Sesquiterpene Lactones* [in Russian], Meditsina, Moscow (1978), p. 20.
3. W. Herz and K. Ueda, *J. Am. Chem. Soc.*, **83**, 1139 (1961).
4. K. M. Turdybekov, S. M. Adekenov, S. V. Lindeman, and Yu. T. Struchkov, *Khim. Prir. Soedin.*, 788 (1989).
5. K. H. Lee, R. F. Simpson, and T. A. Geissman, *Phytochemistry*, **8**, 1515 (1969).
6. S. M. Adekenov, K. A. Aituganov, K. M. Turdybekov, S. V. Lindeman, and Yu. T. Struchkov, *Khim. Prir. Soedin.*, 653 (1991).
7. S. M. Adekenov, N. M. Gafurov, A. Zh. Turmukhambetov, and V. I. Ivlev, *Khim. Prir. Soedin.*, 305 (1987).
8. F. H. Allen, O. Kennard, D. G. Watson, L. Brammer, A. G. Orpen, and R. J. Taylor, *J. Chem. Soc. Perkin Trans. II*, S1 (1987).
9. D. E. Hibbs, J. Overgaard, C. Gatti, and T. W. Hambley, *New J. Chem. (Nouv. J. Chim.)*, **27**, 1392 (2003).
10. M. Parvez, M. Riaz, and A. Malik, *Acta Crystallogr., Sect. E: Struct. Rep. Online*, **57**, 289 (2001).
11. S. M. Kupchan, C. W. Siegel, R. J. Hemingway, J. R. Knox, and M. S. Udayamurthy, *Tetrahedron*, **25**, 1603 (1969).
12. Y.-L. Liu and M. J. Mabry, *Phytochemistry*, **20**, 1389 (1981).
13. H.-J. Seo and Y.-J. Surf, *Mutat. Res.*, **496**, 191 (2001).
14. Y.-R. Deng, A.-X. Song, and H.-Q. Wang, *J. Chin. Chem. Soc.*, **51**, 629 (2004).
15. A. G. Gonzalez, J. Bermejo, A. D. de la Rosa, and G. M. Massanet, *An. Quim. Ser. C*, **72**, 695 (1976).
16. V. Martinez, O. Barbera, J. Sanchez-Parareda, and J. A. Marco, *Phytochemistry*, **26**, 9, 2619 (1987).
17. N. Morita, *Chem. Pharm. Bull.*, **8**, 59 (1960); *Chem. Abstr.*, **55**, 17910 (1961).